Reference: *Biol. Bull.*, **158**: 324–338. (June, 1980)

MORPHOGENIC MOVEMENT AND EXPERIMENTALLY INDUCED DECREASE IN NUMBER OF EMBRYONIC SEGMENTS IN THE JAPANESE HORSESHOE CRAB, *TACHYPLEUS TRIDENTATUS*[1]

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There have been several intensive studies on the normal embryonal development of horseshoe crabs (Packard, 1870, 1872, 1880; Kingsley, 1892, 1893; Kishinouye, 1892; Munson, 1898, 1912; Patten, 1912; Gardiner, 1927; Iwanoff, 1933; Brown and Knouse, 1973; Sekiguchi, 1973; Bennett, 1979), and a few experimental studies of the dense egg masses (Patten, 1894, 1896), by electrocauterization (Oka, 1943; Sekiguchi, 1966) and by cell dissociation (Itow and Sekiguchi, 1979; Itow, 1979). However, the detailed process of the morphogenic movement has remained unrevealed.

In this study, the process of morphogenic movement in the embryo of the horseshoe crab was examined with time-lapse cinemicrographic and histological techniques. In addition, embryos were treated with chemical reagents such as cytochalasin B and dithiothreitol to attempt further elucidation of normal morphogenic movement. In the course of the experiment, monsters showing decrease in number of segments were elicited. Therefore, the characteristics, conditions of induction and process of formation of those monsters were investigated.

The present paper describes the process of morphogenic movement, the induction of monsters, and the relationships between them.

Materials and Methods

Adult Japanese horseshoe crabs, *Tachypleus tridentatus,* collected at the beach at Imari, Saga Prefecture, and Kasaoka, Okayama Prefecture, were brought to the Shimoda Marine Research Center of the University of Tsukuba, Shizuoka Prefecture. There, eggs were artificially inseminated and the fertilized eggs reared in filtered normal sea water in plastic trays at room temperature (24–32° C). The sea water in the trays was exchanged for fresh sea water once or twice a day. The embryos used for observations and experiments were taken from these trays.

The stages of embryonic development were identified by reference to the normal stages described by Sekiguchi (1973).

Cinemicrography

Normal and treated embryos vitally stained with neutral red were filmed using a Nikon 16 mm cinemicrographic apparatus (type CFM–A) at intervals of 1–8 min (usually 4 min). The movements of various parts of the embryos were analyzed by tracing them on the serial cinefilms.

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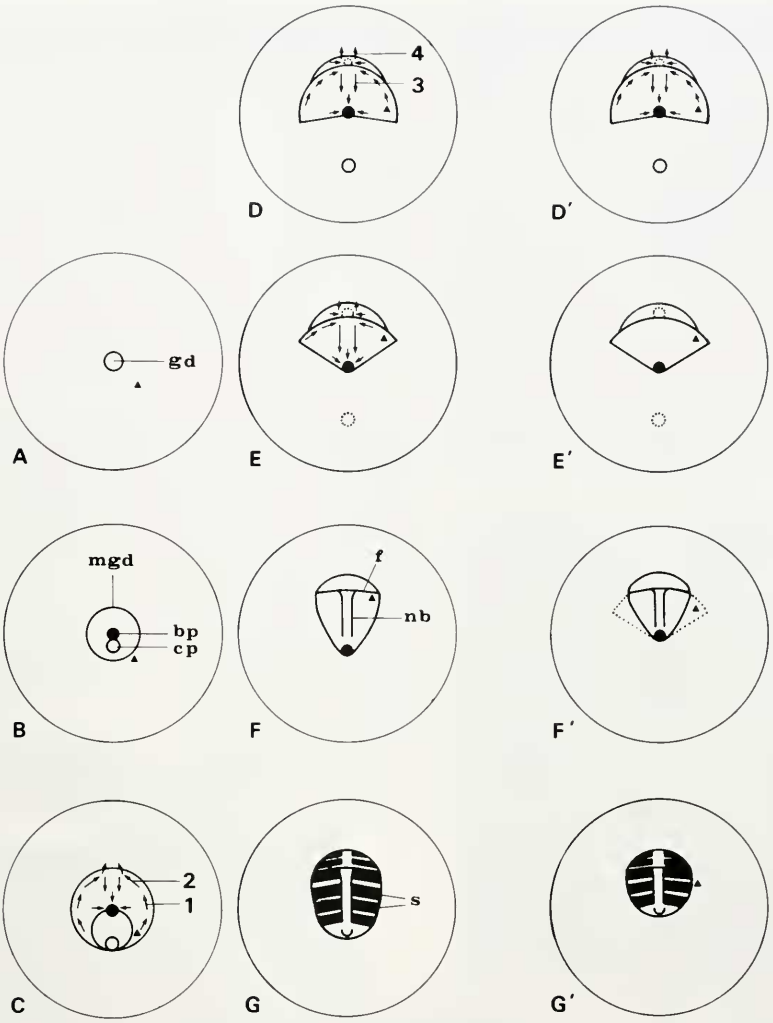

FIGURE 1. Diagrammatic representation of morphogenic movement in normal and segment-defective embryos, drawn from the cinemicrographs.

A. to G. Key morphogenic events in the normal embryo:

A. Germ disc appearance (Stage 7),

B. Spreading of the germ disc (Stage 9),

C. Germ disc completion (Stage 10),

D. Half a day after Stage 10,

E. A day after Stage 10 (Stage 11),

F. End of morphogenic movement (Stage 12),

G. Appearance of segment structure (Stage 13).

D'. to G'. Morphogenic movement in the embryo treated with chemical reagents for 24 hr from stage D.

D'. The stage just after treatment.

Arrows show direction of migration of the surface cells. Solid triangles show points of surface cells traced by cinemicrography. The explanation of 1 to 4 is in the text. bp: blastopore, cp: cumulus posterior, f: region of transverse furrow, gd: germ disc, mgd: margin of germ disc, nb: narrow band, s: segment structure.

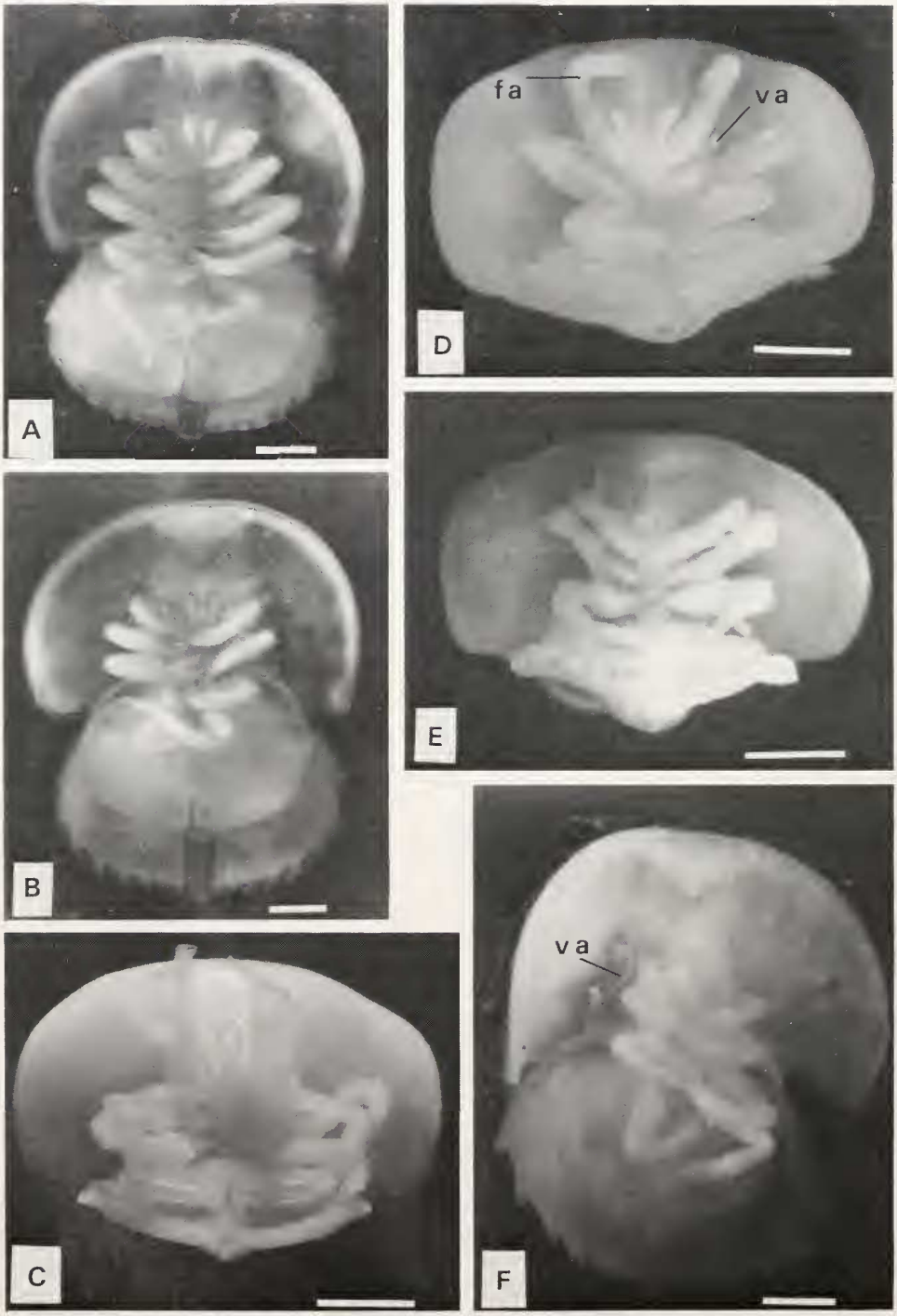

FIGURE 2. Examples of segment-defective embryos.
A. A normal embryo with six pairs of prosomal appendages.
B. A typical segment-defective embryo devoid of the 3rd prosomal segment.

Chemical and physical treatments

Chemical reagents. Many groups of 20 embryos each were cultured separately in small laboratory dishes filled with about 10 ml of natural or artificial sea water containing cytochalasin B (Cyt. B) dissolved in dimethylsulfoxide, or dithiothreitol (DTT) dissolved in distilled water. Other embryos were treated with mercaptoethanol, o-iodosobenzoic acid dissolved in dimethylsulfoxide, $HgCl_2$, arsenite, α-lipoic acid, α-lipoic amide, colchicine, vinblastine sulfate, dimethylsulfoxide and Ca^{2+}-free sea water, respectively. Treatment time varied with the purpose of the experiment. During treatment, the culture media were maintained between 28° and 30° C and renewed every 12 hr. After treatment, the embryos were returned to normal sea water which was also renewed every 12 hr.

Temperature. Embryos at selected stages were exposed to 5°, 37°, or 50° C to observe the effects of low or high temperature on morphogenesis.

Electrocauterization. Specific portions of the embryos were electrically cauterized during morphogenic movement by the method of Oka (1943).

Light and electron microscopy

Normal embryos and embryos treated by the various methods mentioned above were vitally stained with neutral red and observed under a stereoscopic microscope. The histological observations were made by the following light and electron microscopic methods:

1. For light microscopy, normal and treated embryos were fixed in Bouin's, Carnoy's, or FAA (formalin-ethanol-acetic acid, 5:5:1) solutions, embedded in paraffin, and sectioned at 5–20 μm. The sections were stained with Mayer's hematoxylin and eosin, or Azan staining solution.

2. For electron microscopy, specimens were pre-fixed in 5% glutaraldehyde and 4% paraformaldehyde in 8×10^{-2} M cacodilate buffer (*p*H 7.4) for 12 hr, and then post-fixed in 1% osmium tetroxide solution for 2 hr. The sections were stained with saturated uranyl acetate and Reynold's solution.

Results

Morphogenic movement

Migration of cells in the embryo. The results obtained by time-lapse cinemicrography and stereoscopic microscopy are summarized as follows:

The germ disc appeared as a spot on the blastoderm at Stage 7, about 8 days after insemination (Fig. 1-A). The germ disc increased gradually in diameter and reached a maximum at Stage 10 (Fig. 1-C), the stage of germ-disc completion,

C. A segment-defective embryo with right-hand appendages normal, but the 1st to 4th appendages fused on the left side.

D. A segment-defective embryo, with fused 2nd and 3rd appendages and vestigial 4th ones on both sides.

E. A segment-defective embryo obtained by treatment with dithiothreitol for 48 hr. The abnormal region is wide and the 1st to 4th appendages show some abnormalities such as defect or fusion.

F. A segment-defective embryo with a vestigial right 3rd appendage not in line with the five pairs of normal ones.

The embryos of A, B and F were photographed after the 4th embryonic moulting and the embryos of C, D and E after the 3rd embryonic moulting. White bar = 1 mm. fa: fused appendage, va: vestigial appendage.

about 12 days after insemination. During this process, the so-called blastopore appeared at the central portion of the germ disc and the cumulus posterior that appeared at Stage 9 near the blastopore migrated to the inner margin of the posterior end of the germ disc (Fig. 1-B).

Obvious morphogenic movement started in Stage 10. The germ disc began to change its shape. The surface cells of the whole germ disc began to migrate actively. Main migrations of the cells, occurring simultaneously, were as follows (refer to 1–4 in Fig. 1-C and D) : 1.) The cells in the lateral region of the peripheral area of the germ disc migrated to the anterior region. 2.) The cells in the lateral region of the anterior area of the germ disc migrated towards the median body axis of the embryonic area. 3.) The major portion of the cells at the anterior margin of the germ disc moved posteriorly along the median body axis, forming two narrow bands (Fig. 1-F). 4.) Some cells of the anterior margin of the germ disc spread in front of the germ disc.

During these migrations, the blastopore moved to the posterior end of the embryonic area. The embryonic area stretched to double its original length. A transverse furrow was formed at the anterior region of the embryonic area during morphogenic movement. The region where this furrow appeared was identical to the anterior margin of the germ disc at Stage 10 (Fig. 1-C).

Morphogenic movement continued for about two days until Stage 12 (Fig. 1-F), being most rapid at Stage 11 (Fig. 1-E). Segment structures appeared on the embryonic area at Stage 13 (Fig. 1-G). First, the 3rd prosomal segment appeared clearly at the region immediately behind the furrow of the embryonic area, where the peripheral cells of the germ disc gathered actively. The 1st and 2nd prosomal segments appeared in front of this region, and the 4th and the 5th ones appeared behind the 3rd segment. However, the 6th prosomal segment and the opisthosomal segments were not so clear in this stage.

Histological and cytological changes. Some cells larger than the blastoderm cells appeared under the germ disc at Stage 7. These cells, which are not identical with yolk cells, divided actively and began to form the underlayer of the germ disc. After Stage 7, the surface cells of the germ disc changed from flat to cubic, and formed a more compact layer than the other surface cells of the embryo. The reduction in size of the surface cells both inside and outside the germ disc continued uninterrupted from the stage of cleavage on. From insemination to Stage 10, each nucleus of the cells of the surface and the underlayer usually had two nucleoli.

During active morphogenic movement (Stages 10 to 12), the following changes were observed: The cells of the underlayer became smaller and increased in number, and, at the anterior region of the embryonic area, two bands of cells were formed along the median body axis. The surface cells of the embryonic area became cylindrical and more compact. This transformation was especially distinct in the anterior region of the embryonic area. The reduction in size of the cells of the underlayer and surface ended during morphogenic movement. The number of nucleoli in each nucleus decreased, usually from two to one. At Stage 12, microfilaments were observed in the periphery of surface cells at the level of apical junction.

Formation of segment-defective embryos

When the embryos were treated for 24 hr with 5–10 μg/ml Cyt. B or 300–5000 μg/ml DTT during the period of most active morphogenic movement, more than

TABLE I

Frequencies of formation of segment-defective embryos and other monsters by treatment with dithiothreitol and cytochalasin B for 24 hr during active morphogenic movement.

Reagents	No. of normal embryos (%)	No. of segment-defective (%)	No. of other monsters (%)	No. of developed embryos (%)	% of developed embryos per total embryos
Dithiothreitol 500 μg/ml	54 (27.14)	142 (71.36)	3 (1.50)	199 (100.00)	71.1
Cytochalasin B 5 μg/ml	7 (21.21)	26 (78.79)	0 (0.00)	33 (100.00)	66.0
Normal sea water (for control)	2539 (99.57)	1 (0.40)	10 (0.39)	2550 (100.00)	84.3

50% of them formed monsters (Fig. 2, Table I). External and histological observations of these monsters revealed that the defect or fusion of appendages occurred with a simultaneous defect or fusion of the corresponding segments.

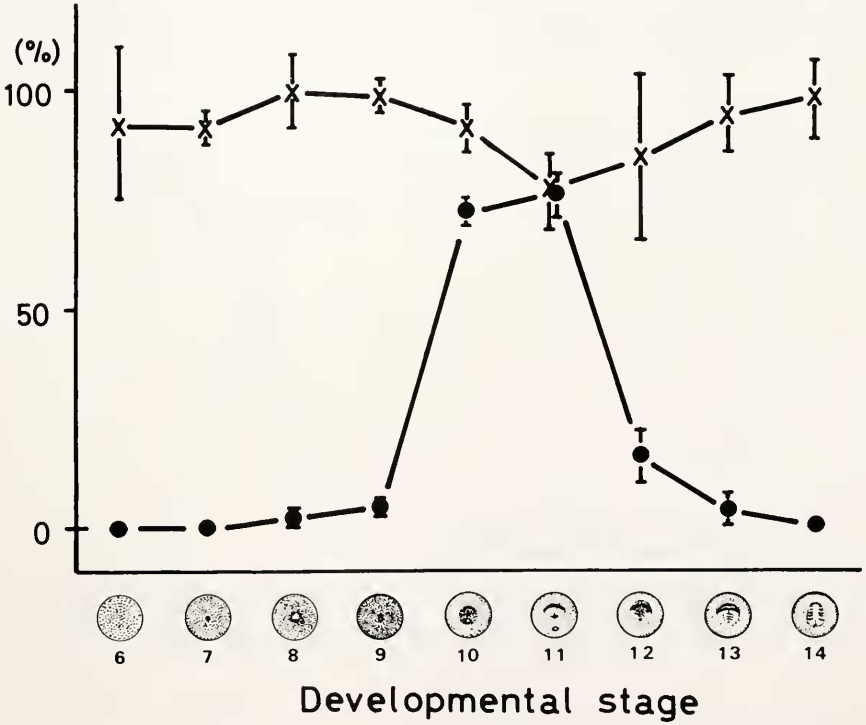

FIGURE 3. Rates of development and formation of segment-defective embryos after 24 hr treatment with dithiothreitol at different developmental stages. Cross: mean rate of development in the treated group as a percentage of the rate in controls. Solid circle: mean rate of formation of segment-defective embryos among developed embryos. Vertical lines with limiting marks: ± 1 standard deviation. The embryos were treated at stages shown in the figure.

These monsters are called segment-defective embryos hereafter. Segment-defective embryos were also induced at a rate higher than 50% by treatment for 24 hr during morphogenic movement with 1.5–3 μg/ml arsenite, 1,300 μg/ml to saturated o-iodosobenzoic acid, 5 μg/ml to saturated α-lipoic acid, 5 μg/ml to saturated α-lipoic amide or 1–2 μg/ml $HgCl_2$. After treatment of the embryos with 7,500–15,000 μg/ml mercaptoethanol, segment-defective embryos were formed at a rate of 0–50%.

The conditions for formation of segment-defective embryos were as follows:

1. Developmental stage. Treatment with the above-mentioned chemical reagents (except Cyt. B) induced segment-defective embryos at any stage during morphogenic movement (Fig. 3). When embryos at Stage 10 were treated with Cyt. B, the death rate became higher and few segment-defective embryos were induced (Fig. 4). With this reagent, monsters were induced only when the embryos were treated at Stage 11 (1 day after Stage 10). When the embryos were treated with the above-described reagents only before or after morphogenic movement, segment-defective embryos were not induced.

2. Treatment time. Embryos treated with the above-mentioned chemical reagents for 24 hr formed segment-defective embryos at the highest rate. They were formed at nearly the same rate with treatment for only 12 hr at Stage 11.

3. Concentration of chemical reagents. When treated for 24 hr with DTT during morphogenic movement, embryos treated with less than 200 μg/ml DTT developed normally and embryos treated with more than 10,000 μg/ml DTT

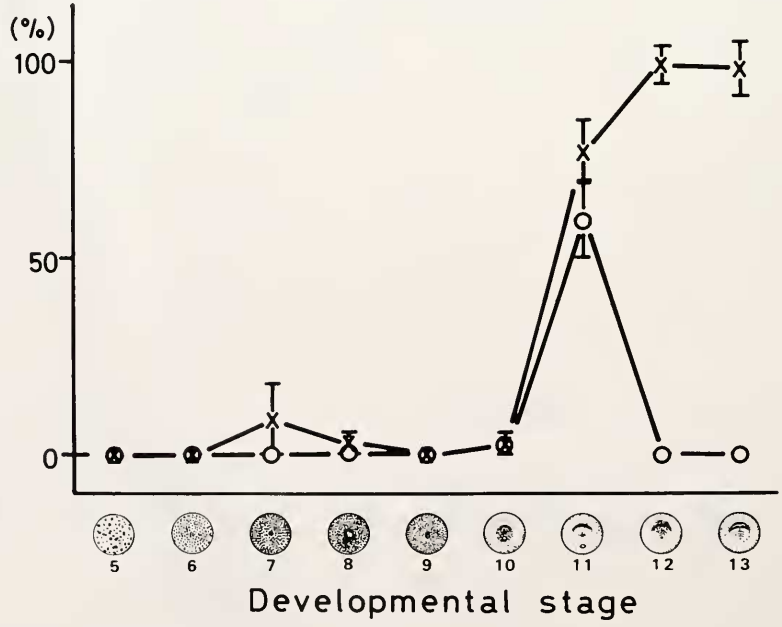

FIGURE 4. Rates of development and formation of segment-defective embryos after 24 hr treatment with cytochalasin B at different developmental stages. Cross: mean rate of development in the treated group as a percentage of the control rate. Open circle: mean rate of formation of segment-defective embryos as a percentage of developed embryos in the control experiment. Vertical line with limiting marks: $\pm$ 1 standard deviation. Embryos were treated at the stages shown in the figure.

stopped developing. Concentrations between these caused segment-defective embryos. When embryos at Stage 10 were treated with Cyt. B for 24 hr, embryos treated with more than 5 μg/ml Cyt. B did not develop and those treated with less than 2 μg/ml Cyt. B developed normally. With treatment for 24 hr with Cyt. B at Stage 11, embryos treated with less than 2 μg/ml Cyt. B developed normally and embryos treated with more than 20 μg/ml Cyt. B did not develop. Concentrations between these caused segment-defective embryos.

4. Other reagents. When embryos were treated with Ca^{++}-free sea water for 24 hr during morphogenic movement, various types of monsters were formed. Segment-defective embryos were included in these, but the rate was lower than 25% of developed embryos. Colchicine, vinblastine sulfate, and dimethylsulfoxide failed to induce segment-defective embryos or any other monsters. That is, when embryos were treated for 24 hr during morphogenic movement with less than 10^{-1} μg/ml colchicine, less than 1 μg/ml vinblastine sulfate or less than 5 μg/ml dimethylsulfoxide, they developed normally. When embryos were treated for 24 hr during morphogenic movement with more than 1 μg/ml colchicine or 10 μg/ml vinblastine sulfate, they did not develop.

5. *Temperature.* Treatment with low or high temperature failed to produce segment-defective embryos. When embryos were cultured for 24 hr at 5° or 37° C during morphogenic movement, they developed normally. With treatment at 50° C, embryos did not develop.

Characteristics of segment-defective embryos

The segment-defective embryos obtained by treatment with chemical reagents such as Cyt. B and DTT showed the following characteristics: (No appreciable difference was recognized between the effects of the different reagents.)

Most of the segment-defective embryos showed an unsymmetrical defect or fusion of appendages.

Fusion was observed between the anterior and the posterior appendages of the right or left side, but not between the right and the left sides.

The occurrence of abnormal appendages is shown in Figure 5. About 50% of the examined sides having abnormal appendages showed the defect in only one appendage. As the appendages between the 2nd and the 5th have similar forms, the difference between appendages is not clear at first sight and the appendage cannot always be confirmed. Therefore, the structure and size of the appendages of segment-defective embryos with five pairs of prosomal appendages were compared with those of normal embryos. It was revealed that the 3rd prosomal appendages in these monsters disappeared and the other appendages were hardly different from normal ones (Fig. 6). In addition, it was found that some embryos having only four pairs of prosomal appendages were devoid of the 3rd and the 4th appendages.

In some of the segment-defective embryos, a vestigial 3rd prosomal appendage was observed outside the normal row of appendages (Fig. 2-F).

In cases of fused appendages, the fusion between the 3rd and the 4th prosomal appendages was most frequent, followed by fusion between the 2nd and the 3rd appendages.

In all segment-defective embryos, the 5th and 6th prosomal appendages as well as all the opisthosomal ones were always normal (Fig. 5).

From these results, it may safely be said that abnormality of appendages occurs mainly in the 3rd prosomal segment.

Relationships between the conditions of induction and the types of abnormality of appendages were as follows:

No significant difference was observed in the types of defects following treatment at different developmental stages.

With treatment for less than 24 hr, embryos devoid of one appendage were fewer and embryos with one or two fused appendages were more common, compared with 24-hr treatment. When treatment time was longer than 24 hr, the abnormal area became wider and various types of defects and fusion were observed in the 1st to the 4th prosomal appendages (Fig. 2-E).

When embryos were treated with lower than 500 μg/ml DTT or 5 μg/ml Cyt. B, embryos having fused appendages outnumbered those devoid of one appendage. In concentrations higher than 500 μg/ml DTT or higher than 5 μg/ml Cyt. B, embryos lacking one appendage on one or both sides outnumbered embryos with fused appendages. With these treatments, however, the abnormality of appendages was limited almost entirely to the 3rd prosomal segment, and the abnormal area did not become wider.

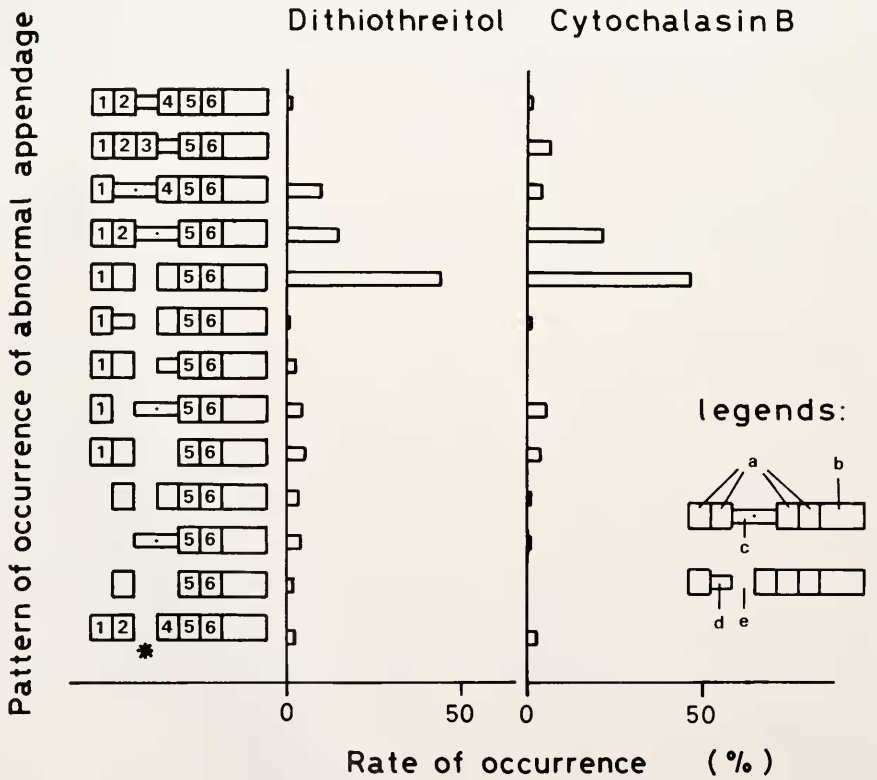

FIGURE 5. The pattern and rates of occurrence of abnormal appendages induced with dithiothreitol and cytochalasin B. a: normal prosomal appendages. b: normal opisthosome (abdomen). c: a vestigial appendage. d: fused abnormal appendages. e: missing appendages. But the 2nd to 5th appendages of the horseshoe crab embryos have similar forms, so the missing appendages are not always clear. *: the pattern showing a vestigial 3rd prosomal appendage not in line with other appendages. 1 to 6: These numerals show the appendages having the same structure as the 1st to 6th prosomal ones of normal embryos.

Characteristics of morphogenesis in embryos treated with reagents

When embryos were treated with chemical reagents effective in inducing segment-defective embryos, morphogenic movement stopped almost completely during treatment (Figs. 1, 7). When these embryos were returned to normal sea water, morphogenic movement started again. However, both migration of the peripheral cells of the germ disc and elongation of the embryonic area ended without their attaining normal position and length, and the segment structures appeared successively. These embryos developed into embryos showing abnormality in or near the 3rd prosomal segment.

Morphogenic movement also stopped during exposure to low temperature (5° C). However, these embryos developed normally when returned to an incubator at 28°–30° C. Morphogenic movement and the elongation of the embryonic area started again and proceeded normally.

Histological observations revealed phenomena common to embryos treated with chemical reagents and those cultured at low temperature. In both, the cells in the underlayer were prevented from becoming smaller, increasing in number, or gathering. In addition, the transformation of the surface cells of the embryo into cylindrical shape was stopped. However, the decrease in the number of

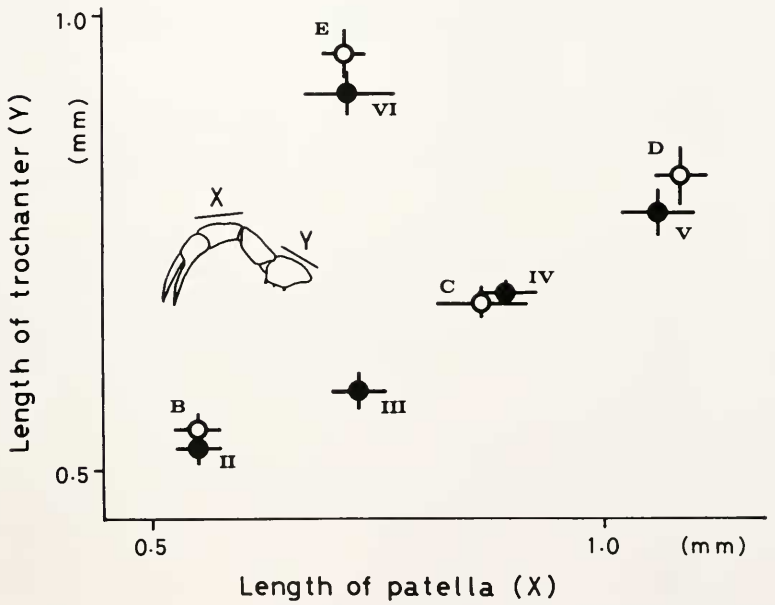

FIGURE 6. Comparison of the length of two portions of the appendages in normal embryos and segment-defective ones having five pairs of prosomal appendages at hatching stage. (Each appendage of 30 normal embryos and 13 segment-defective embryos was cut out and measured with micrometer under the microscope. The unit of measurement was 10 or 25 μm.)

Closed circle: normal embryo. Open circle: segment-defective embryo. Vertical and horizontal bars: ± 1 standard deviation.

II to VI: 2nd to 6th prosomal appendages in the normal embryo. B to E: 2nd to 5th prosomal appendages of segment-defective embryos from anterior to posterior. The 5th appendages or the last prosomal appendages of these defective embryos have the same structure as the 6th ones or the last prosomal ones of normal embryos. In short, this type of segment-defective embryo lost only the 3rd prosomal segment.

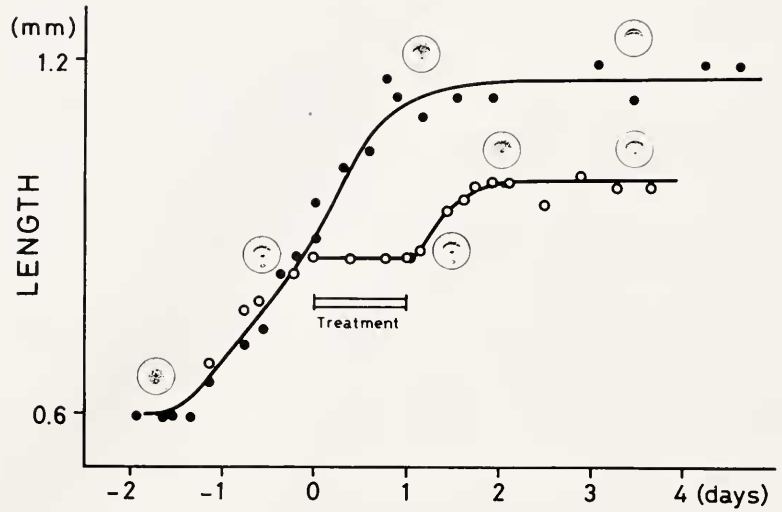

FIGURE 7. Elongation of the embryonic area observed by cinemicrography in normal and treated embryos. Closed circle: length of embryonic area in normal embryos. Open circle: length of embryonic area in an embryo treated with dithiothreitol for 24 hr at the period shown. The treated embryo developed lacking the 3rd prosomal appendage in the right side and with fused 3rd and the 4th appendages on the left. Length of embryonic area was measured between the anterior end and the blastopore.

nucleoli differed between these two treatments. The number of nucleoli decreased with treatment by chemical reagents, but not with culture at 5° C. When embryos were treated with chemical reagents, no enucleated or polynucleated cell was observed.

By electron microscopic observations, a difference in the adhesion of surface cells was recognized between normal embryos and embryos treated with chemical reagents. In normal embryos, the cells showed complete adhesion. However, in embryos treated with DTT or Cyt. B, adjacent cells were partly separated. Microfilaments were observed at the apical circumferences of the cylindrical surface cells in the normal embryo, but they could not be observed in the cubic surface cells of the normal embryo or in the cubic cells whose transformation into cylindrical shape was stopped with chemical reagents. On the other hand, microtubules were observed even in the treated embryos. In embryos treated with chemical reagents, secretion of the inner egg membrane and transformation of the surface cells were stopped in the same state as before treatment. Organelles such as mitochondrion, Golgi body, ribosome, nucleus and cell membrane showed no recognizable difference between the normal and the treated embryos.

By histological observations, no appreciable difference in histological features was recognized between hatched larvae cultured in normal sea water after being returned from the treatment solution and normal larvae, although a slight retardation of development was observed in the treated embryos.

Formation of segment-defective embryos by electrocauterization

Segment-defective embryos were also formed by electrically cauterizing the same region in which cell migration to the normal position was inhibited by chemical treatment.

The position indicated by the black triangle in Figure 1-E and the adjacent region were cauterized in 57 embryos. Of these 57 embryos, 34 developed. Fourteen of them were segment-defective embryos. The defects and fusion were observed at the 3rd prosomal segment and the neighboring segments in the left side of embryos. The abnormal segments were much the same as those in the monsters obtained by treatment with chemical reagents.

Discussion

It has been known that in some Vertebrata and Echinodermata morphogenic movement can be disturbed by chemical reagents such as Cyt. B and DTT, and that various types of malformations such as incomplete formation of archenteron and neural tube are induced (Runnström and Kriszat, 1952; Runnström, 1956; Brachet, 1959; Seilern-Aspang, 1959; Laskshmi, 1962; Wessells *et al.*, 1971; Schaeffer and Brick, 1972; Brachet and Tencer, 1973).

In the present study, the authors observed the processes of morphogenic movement and histological changes of the embryonic area during morphogenic movement in certain developmental stages of the horseshoe crab (Fig. 1). The study revealed that morphogenic movement of the horseshoe crab can be disturbed by treatment with Cyt. B or DTT, and that more than 50% of the treated embryos develop into segment-defective embryos (Table I; Fig. 2). Mercaptoethanol, o-iodosobenzoic acid, arsenite, $HgCl_2$, α-lipoic acid and α-lipoic amide also caused segment-defective embryos at a high rate. This implies that such reagents prevent embryos from completing morphogenic movement, since they are generally thought to be inhibitors of cell movement via disturbance of SH-SS interchange or microfilament formation.

The authors tried several kinds of experiments to test this possibility and obtained the following results showing a positive relationship between experimental induction of segment-defective embryos and disturbance of morphogenic movement: The optimal time for experimental induction of segment-defective embryos coincided well with the period of morphogenic movement (Figs. 3, 4). Cinemicrographic observation showed that morphogenic movement ceased completely under treatment with the chemical reagents, and that after treatment it proceeded normally again but stopped before attaining the final state of morphogenic movement in normal embryos (Figs. 1, 7). The segment-defective embryos thus obtained commonly also had lost the 3rd prosomal segment (Figs. 5, 6). The location of the prospective region which takes part in the formation of the 3rd prosomal segment was confirmed by electrocauterization experiments, which obtained the same type of segment-defective embryo as was induced with chemical reagents. Since the 3rd prosomal segment may originate from cells which migrate from the periphery of the germ disc to the region just behind the furrow of the anterior embryonic area, incomplete morphogenic movement probably brings about the exclusion of the cells prospective for the 3rd prosomal segment (Fig. 1). Embryos having a small vestigial 3rd prosomal appendage located outside a normal row of other appendages are thought to be a result of disturbance of morphogenic movement due to the chemical reagent treatment (Fig. 2-F). The defective area became wider with treatment longer than 24 hr. This may be explained by an increase of the excluded area as a result of less complete morphogenic movement.

The mechanism inducing incomplete morphogenic movement has, so far, been unclear. However, we can speculate that incomplete morphogenic movement might arise from dissociation of coordination between cell movement and morphogenesis. In normal development, of course, they would be coordinated. With low-temperature treatment, both may be stopped simultaneously during treatment, and treated embryos may develop normally after they are transferred to 28–30° C. Coordination may be dissociated by chemical reagent treatment. It seems to be evidence of dissociation of coordination that the decrease of nucleolus number was not accordant with morphogenic movement in embryos treated with chemical reagents. If the formation of segment structure begins during the delayed cell movement, the cells of a region could be excluded.

Analysis of experimentally induced segment-defective embryos gives us some information on the mechanism of normal morphogenic movement, segmentation and appendage formation. Body segmentation can occur even when morphogenic movement is incomplete. The 3rd prosomal segment seems not to be necessary for normal development of the other segments or appendages, since other appendages of embryos devoid of the 3rd prosomal appendage had the same structure and size as those of the normal embryo (Fig. 6). However, the region near the furrow of the anterior embryonic area may be important for morphogenesis, since morphogenic movement is most active there. Some appendages and segments may be determined to some extent at stages before morphogenic movement, because, in spite of defects of some segments, other segments and appendages of an embryo form normally. Abnormalities such as defects or fusion of appendages occur independently in the left and the right sides of the same segment-defective embryo. It is likely, therefore, that the migration of cells on each side of the embryonic area and the formation of segments of each side proceed independently to a certain extent.

Morphogenic movement in the horseshoe crab shows the same pattern as seen in the Entelegynae of the Araneida in which "Umkippen" occurs (Holm, 1952). This result is inconsistent with the fate map depicted by Anderson (1973) from the study of Iwanoff (1933) but consistent with the results of Sekiguchi (1973).

The authors thank Prof. Hiroshi Watanabe, the director, Prof. Arinobu Ebara, the former director, and other members of the Shimoda Marine Research Center for their hospitality.

Summary

Active morphogenic movement in the horseshoe crab, *Tachypleus tridentatus,* was observed during the 2 days following completion of the germ disc.

Main migration of cells is as follows: Peripheral cells of the germ disc migrate to the anterior margin of the germ disc, and the cells of the anterior margin of the germ disc migrate posteriorly along the median body axis. During such migrations, the transverse furrow appears at the anterior margin of the germ disc.

The 3rd prosomal segment is formed from the region just behind the furrow.

By treatment of embryos with chemical reagents such as cytochalasin B, dithiothreitol and α-lipoic acid, morphogenic movement was disturbed and segment-defective embryos were formed.

Defect or fusion of appendages and segments in the segment-defective embryos occurred mainly in the 3rd prosomal segment. A possible explanation is that the prospective region forming the 3rd prosomal segment cannot take part in the formation of segments as a result of the inhibition of morphogenic movement by chemical reagents.

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